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# Evaluate Ethanol Extracts of Plant Extract in Animal Models of Anxiolytic Potential

**Yerra Keerthi Reddy*, D. Swathi, V. Umarani, K Shravan Kumar**

*Samskruti College of Pharmacy, Kondapur, Ghatkesar, Medchal, Hyderabad-501301, Telangana, India*
***Corresponding E-Mail:** principal.y7@gmail.com*

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**Abstract:**
Currently, approximately one-fifth of the adult population worldwide is expected to experience an anxiety disorder at some point in their lifetime. Over the past decades, globalization of information has increased public awareness of the side effects associated with conventional psychopharmacological treatments, such as sedation, drowsiness, and withdrawal symptoms. This has driven many individuals in Western societies to explore alternative therapies, including self-administered herbal remedies. The use of plant-based treatments has grown significantly and is no longer limited to developing countries, resulting in a rising demand for medicinal plant products in Western nations. Despite the increasing popularity of natural health products, pharmaceutical research in this field has declined over the past decade due to the complexity, time consumption, and limited marketability of such studies. Expanding scientific research in phytotherapy with empirical evidence could improve clinical applications and industrial feasibility. This dissertation aims to evaluate the anxiolytic potential of Valeriana officinalis roots. Plant-derived natural products have been used for centuries to treat various ailments, and Valeriana officinalis roots are traditionally employed for memory enhancement, as a brain tonic, and as a hepatoprotective agent. While the leaves and seeds of the plant have demonstrated nootropic and anticonvulsant activity, the roots were specifically investigated in this study for their effects on anxiety and exploratory behavior in animal models. These findings highlight the need for further investigation into the neuropharmacological mechanisms underlying the anxiolytic effects of V. officinalis. Future studies should explore receptor binding of specific chemical constituents to determine their affinities for neurotransmitter systems involved in anxiety. Continued research into herbal anxiolytics could lead to the development of safe, effective, and economically feasible alternatives to conventional therapies. However, standardization of herbal products remains a critical challenge limiting their widespread clinical use.

**Keywords:** *Anxiolytic Potential, Valeriana, hepatoprotective agent, phytotherap*

## Introduction

### Anxiety and Its Modeling

Anxiety is an adaptive response that enables individuals to recognize and cope with potential threats. Fear differs from anxiety in that it represents a reaction to a known, specific danger, whereas anxiety arises from vague or uncertain threats. Clinically, anxiety is classified as either

**Normal anxiety –** an appropriate, beneficial response to real-life challenges

**Pathological anxiety –** an exaggerated or inappropriate response to external or internal stimuli

Given the complexity of anxiety disorders and their frequent comorbidity with depression, creating comprehensive models that fully reflect human pathology is difficult. Most experimental paradigms expose animals to external stimuli (e.g., foot-shock cues, bright light, predator exposure) or internal stimuli (e.g., anxiogenic drugs). These generally induce state anxiety, i.e., a temporary anxiety-like condition, rather than pathological anxiety.

Despite limitations, animal models remain indispensable for neurobiological and neuropharmacological research. They have provided crucial insights into the etiology, physiology, and treatment responses of fear and anxiety.

### Models for Normal Anxiety

**Categorized these models into two groups:** Unconditioned response-based models Conditioned response-based models

**1. Elevated Plus Maze (EPM)**

The EPM is a widely used, simple method to assess rodent anxiety. It exploits rodents' natural preference for enclosed, dark spaces (approach) versus their innate fear of open, elevated spaces (avoidance).

**Procedure:** Rodents are placed at the intersection of four arms (two open, two closed) and observed for 5 minutes. Behavior is recorded as time spent and entries into open versus closed arms.

**Interpretation:**

More open-arm entries and time = anxiolytic effect. Preference for closed arms = anxiety-like behavior

**Applications:**

Screening novel anxiolytic drugs Assessing effects of stressors (prenatal/postnatal).Exploring brain mechanisms underlying anxiety.

**Staircase Test:** In this model, a mouse is placed in an enclosed staircase with five steps. The number of steps climbed and rearings within 3 minutes are recorded.

**Findings:** Anxiolytics (e.g.,diazepam,lorazepam, phenobarbital) reduce rearing without affecting step climbing.

Non-anxiolytic drugs (e.g., haloperidol, morphine) reduce both behaviors, lacking anxiolytic specificity.

**Conclusion:** Increased climbing with reduced rearing indicates anxiolytic activity.

**3. Rotarod Test:** Although primarily used to assess motor coordination and cerebellar function, the rotarod test can

distinguish between motor deficits and non-motor symptoms in rodents

**Significance:** Mice with dopamine depletion show minimal deficits, making the test valuable for studying non-motor, anxiety-related behavioral changes. Modified versions assess motor learning versus maximal performance.

**Models for Pathological Anxiety**

Pathological anxiety is often persistent, with genetic predispositions playing a key role. To model this, genetically modified mice or selectively bred rodent lines are used.

**Genetic Models**

**5-HT1A receptor knockout mice**

Null mutants across multiple strains (C57BL/6, 129/Sz, Swiss-Webster) consistently showed increased anxiety, supporting the critical role of serotonin (5-HT) in anxiety regulation.

**CRF and ABAA receptor mutants**

Knockouts for corticotrophin-releasing factor (CRF) or the γ2 subunit of the GABAA receptor displayed strong anxiety phenotypes. The γ2 subunit is essential for benzodiazepine-mediated anxiolysis.

Neuropeptide Y (NPY) knockout mice

Lack of NPY results in heightened anxiety behaviors.

Selective Breeding Models

BALB/c mice: exhibit trait anxiety due to abnormalities in GABAergic, dopaminergic, and opioid systems

Rat strains: Wistar-Kyoto, Roman, Maudsley, and Sardinian alcohol-preferring lines display high baseline anxiety.

HAB/LAB rats: selectively bred Wistar rats show high (HAB) or low (LAB) anxiety-related behaviors. HAB rats exhibit stronger stress responses, linking innate emotionality with coping mechanisms.Animal models are indispensable tools in understanding the neurobiology of anxiety and depression, as well as in developing novel anxiolytic and antidepressant drugs. While no single model fully replicates human pathological anxiety, the use of multiple models—employed either sequentially or in parallel provides the best opportunity to unravel underlying mechanisms and identify effective therapies.

## Materials and Methods

**Test Materials**

**Test drug:** Valeriana officinalis root extract

Standard drug: Diazepam (Dr. Reddy's, Hyderabad)

**Chow diet:** Rayan Biotech Pvt. Ltd., Hyderabad

**Animals and Housing Conditions**

Wistar albino mice were selected as the experimental model, as they are widely used in reproductive and developmental toxicity studies with available historical control data.

**Source**:

Healthy young adult male and female mice were procured from Mahaveer Enterprises (Reg. No: 1362/C/10/ CPCSEA), Hyderabad.

**Randomization**:

Animals were randomized and allocated to treatment groups.

**Body weight**: Only mice with uniform body weights (deviation <20% of mean) were included.

**Acclimatization**:

Animals were acclimated for 5 days prior to the start of the study.

**Housing**:

Mice were housed individually in suspended stainless steel cages, except during acclimation, mating, and nursing. Pregnant females were housed separately.

**Diet and water**: Basal diet and filtered tap water were provided *ad libitum*.

**Environmental conditions**: Temperature: 22 ± 3 °C,Humidity: 50 ± 20%, Light/Dark cycle: 12 h (light: 8:00–20:00, dark: 20:00–8:00), Ventilation: 10–15 air changes per hour

Animal Diet Composition (Rayan Feeds Ltd., Hyderabad) Protein: 22.28%, Total oil: 5.58%

Dietary fiber: 3.86%,Moisture: 9.12%,Salt: 0.13,Ash: 3.69%

**Collection and Authentication of Plant Material**

The rhizomes of *Valeriana wallichii* were collected in June from Bangalore, Karnataka, India. The plant material was authenticated by Dr. Satyanarayana, Scientist, Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

**Preparation of Plant Extracts**

***Shade Drying and Powdering:*** Rhizomes were shade-dried, powdered using a mechanical grinder, sieved (#22), and stored in an airtight container until use.

**Ethanolic Extract (Maceration Method)**

150 g of powdered *Valeriana officinalis* was soaked in 500 ml of ethanol for 2 weeks.The mixture was stirred regularly and filtered through muslin cloth and Whatman No. 1 filter paper.The filtrate was concentrated using a rotary evaporator (Equitron Roteva) at 50–60 °C under reduced pressure.The extra was further dried on a water bath at 50 °C and stored at 4 °C.

***Ethanolic Extract (Soxhlet Method)***

50 g of powdered material was extracted with ethanol using a Soxhlet apparatus for 48 h.

The extract was concentrated, evaporated to dryness in a vacuum desiccator, and stored at 4 °C.The yield was found to be 11% w/w.

**Preliminary Phytochemical Screening**

All extracts of *V. officinalis* were tested for active constituents using standard reagents:

**Alkaloids**: Dragendorff's reagent, Mayer's reagent

**Steroids**: Libermann–Burchard's reagent, Salkowski test

**Carbohydrates**: Fehling's test (A & B), Benedict's reagent, Molisch's reagent

**Saponins**: Frothing test with olive oil emulsion

**Terpenoids**: Chloroform–sulfuric acid test **Amino acids**: Ninhydrin test

**Experimental Models**

General Behavioral Profile (Irwin Test)

Twenty adult albino mice were divided into two groups:

**Test group:** Received ethanolic extract (500 mg/kg, p.o.)

Control group: Received acacia suspension (2 ml, p.o.)

**Animals were observed for behavioral changes:**

Observation intervals: Every 30 min in the first hour, then hourly for the next 6h.

**Parameters assessed**

Awareness and alertness (scored 0–4) Stereotyped movements (e.g., head searching, circling, self-biting, tail lashing; scored 0–1) Spontaneous activity (scored 0–4 in bell jar and on table) Touch response (tested with pencil/forceps) Pain response (tail clamp) Vocalization (normally absent in albino mice)Reflexes: pinna, corneal, and ipsilateral flexor reflex.

**Fig. 1**: Behavioural changes in mice

**Fig. 2**: Behavioural changes in mice

## Result and Discussion

**Table 1:** Effect of ***A. brasiliana*** Extract on Fasting Blood Glucose Levels in STZ-Induced Diabetic Mice

| Evaluation parameters | GROUP 1 scores | GROUP-2 scores | GROUP-3 scores | CONTROL scores | STANDARD scores |
|---|---|---|---|---|---|
| **Alertness** | 3 | 3.5 | 3 | 4 | 2 |
| **Grooming** | 0 | 0 | 0 | 0 | 0 |
| **Passivity** | 0.5 | 0 | 0 | 0 | 0 |
| **Reactivity** | 3.5 | 4 | 4 | 4 | 3 |
| **Pain response** | 4 | 4 | 4 | 4 | 3 |
| **Touch response** | 4 | 4 | 4 | 4 | 3 |
| **Limb position** | 4 | 4 | 4 | 4 | 3 |
| **Body position** | 4 | 4 | 4 | 4 | 3 |
| **Corneal reflex** | 4 | 4 | 4 | 4 | 3 |
| **Righting reflex** | 0 | 0 | 0 | 0 | 1 |

**Table 2**: Table 2: Effect of valerian officianlis roots extracts on Rotarod test.

| Groups | Treatment | Fall off time in sec |
|---|---|---|
| 1 | Control | 304 ± 2.9 |
| 2 | Extract soluble in acetone(500mg/kg) | 53.6 ±2.7* |
| 3 | Extract insoluble in acetone(500mg/kg) | 101.7 ±5.5* |
| 4 | petroleum ether extract(250mg/kg) | 307.4 ± 4.6 |
| 5 | Standard (diazepam 2mg/kg) | 34.6± 2.6* |

All values are Mean ± SEM of n=6, One way ANOVA followed by Brown forsythe test, Bartlett's test *p<0.05 when compared to control animal.

**Table 3:** Effect of valerian officianlis roots extracts on Elevated plus maze test.

| Groups | Treatment | No. of entries into open arm | Open arm time in sec |
|---|---|---|---|
| **1** | **Control** | 6.8 ± 0.4 | 63.33 ± 3.33 |
| **2** | **Extract soluble in acetone 500mg/kg** | 12.4 ± 0.33* | 173.3 ± 6.66* |
| **3** | **Extract insoluble in acetone(500mg/kg)** | 10.8 ± 0.574* | 113.3 ± 6.66 |
| **4** | **petroleum ether extract(250mg/kg)** | 5.4 ± 0.4 | 58.3 ± 4.4 |
| **5** | **Standard (diazepam 2mg/kg)** | 15.6 ± 0.3* | 267.4 ± 1.528* |

***Table 4: Effect of valerian officianlis roots extracts on Stair case test:***

| Groups | Treatment | No. of rearings | No. of steps climbed |
|---|---|---|---|
| **1** | **Control** | 20.33 ± 0.8 | 16.64 ± 0.57 |
| **2** | **Extract soluble in acetone 500mg/kg** | 12.67 ± 0.66* | 10.62 ± 0.3* |
| **3** | **Extract insoluble in acetone(500mg/kg)** | 15.67 ± 0.66 | 13.8 ± 0.57 |
| **4** | **petroleum ether extract(250mg/kg)** | 19.62 ± 0.57 | 16.84 ± 0.54 |
| **5** | **Standard (diazepam 2mg/kg)** | 8.66 ± 0.33* | 7.6± 0.33* |

### Discussion

Currently, approximately one-fifth of the adult population worldwide is expected to experience an anxiety disorder at some point in their lifetime. Over the past decades, globalization of information has increased public awareness of the side effects associated with conventional psychopharmacological treatments, such as sedation, drowsiness, and withdrawal symptoms. This has driven many individuals in Western societies to explore alternative therapies, including self-administered herbal remedies. The use of plant-based treatments has grown significantly and is no longer limited to developing

countries, resulting in a rising demand for medicinal plant products in Western nations. Despite the increasing popularity of natural health products, pharmaceutical research in this field has declined over the past decade due to the complexity, time consumption, and limited marketability of such studies. Expanding scientific research in phytotherapy with empirical evidence could improve clinical applications and industrial feasibility.

This dissertation aims to evaluate the anxiolytic potential of Valeriana officinalis roots. Plant-derived natural products have been used for centuries to treat various ailments, and Valeriana officinalis roots are traditionally employed for memory enhancement, as a brain tonic, and as a hepatoprotective agent. While the leaves and seeds of the plant have demonstrated nootropic and anticonvulsant activity, the roots were specifically investigated in this study for their effects on anxiety and exploratory behavior in animal models.

In preliminary studies, the petroleum ether extract of V. officinalis showed no significant CNS effects, whereas the ethanolic extract exhibited noticeable activity, likely due to the presence of flavonoids, triterpenoids, and steroids. The ethanolic root extracts significantly influenced spontaneous activity, reactivity, and passivity, producing depressive effects on awareness and alertness. However, reflex responses such as righting, corneal, and vocalization remained unaffected throughout the study, indicating selective sedative and anxiolytic actions. Reduced reactivity and suppression of irritability under stress suggest anxiolytic properties of the extracts. Observations using Irwin's table further confirmed the sedative and anxiolytic effects, prompting subsequent behavioral tests.

The Elevated Plus Maze (EPM) test, a widely accepted preliminary screen for behavioral modulation in rodents, was employed to evaluate anxiolytic activity. This test leverages rodents' natural conflict between exploring open spaces and seeking enclosed areas. Open arms of the maze, considered aversive due to perceived predatory risk, are particularly sensitive to GABAergic modulation. In the study, standard diazepam significantly increased both entries and time spent in the open arms, as expected. Similarly, administration of 500 mg/kg of the ethanolic extract markedly increased open-arm exploration while decreasing time in closed arms, indicating potent anxiolytic activity likely mediated through GABA_A receptor modulation.

The RotaRod test was used to assess potential sedative or neurotoxic effects and motor coordination deficits caused by CNS-active compounds. A significant reduction in fall time from the rotating rod was observed in mice treated with 500 mg/kg ethanolic extract and diazepam, reflecting muscle relaxation and a calming effect, which may contribute to reduced anxiety. The Staircase test, which measures exploratory and locomotor activity through the number of steps climbed and rearings, provided additional evidence of anxiolytic effects. Ethanol extract-treated animals exhibited a significant reduction in rearing compared to controls, consistent with anxiolytic activity.

These findings highlight the need for further investigation into the neuropharmacological mechanisms underlying the anxiolytic effects of V. officinalis. Future studies should explore receptor binding of specific chemical constituents to determine their affinities for neurotransmitter systems involved in anxiety. Continued research into herbal anxiolytics could lead to the development of safe, effective, and economically feasible alternatives to conventional therapies. However, standardization of herbal products remains a critical challenge limiting their widespread clinical use.

## Conclusion

The present study demonstrates that the ethanolic root extracts of Valeriana officinalis possess significant anxiolytic and sedative properties in experimental animal models. The extracts produced marked effects on behavioral parameters, including reduced reactivity, suppression of irritability, and enhanced exploratory activity, consistent with anxiolytic activity. In the Elevated Plus Maze test, the extracts significantly increased open-arm entries and time spent, paralleling the effects of diazepam, suggesting modulation of the GABAergic system. In the Rotarod test, reductions in fall-off time reflected sedative and muscle relaxant activity, while in the Staircase test, decreases in rearing behavior further supported anxiolytic potential. Collectively, these findings validate the traditional use of V. officinalis roots in the management of anxiety and related disorders. However, standardization of the extracts and further mechanistic studies are warranted to isolate the active constituents, determine their receptor-level interactions, and establish long-term safety profiles. Such evidence could pave the way for developing safe, effective, and plant-based alternatives to conventional anxiolytic drugs

## Conflict of Interests

The authors declare no conflict of interest

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## AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

## Data Availability

Data will be available on request

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